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Austrolebias arachan (Cyprinodontiformes, Rivulidae), a new species of annual fish from northeastern Uruguay

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***Austrolebias arachan* (Cyprinodontiformes, Rivulidae), a new species of annual fish from northeastern Uruguay**. - The annual fish *Austrolebias arachan* is described from temporary ponds of Río Tacuarí (Los Patos-Merín system) and Río Negro (Río Uruguay drainage) basins. *Austrolebias arachan* is easily distinguished from other *Austrolebias* species by the combination of the following characters: body of males dark brown to black, with light narrow yellow vertical bands, dorsal fin dark brown with vertically elongated light yellow dots in the proximal half, pectoral fin light grey with margin black; females with anterior anal fin rays elongated, forming a triangular-shaped fin. Both sexes with pelvic fins joined to each other in different degrees; two to four parietal neuromasts; three pectoral radials in most individuals. The new species is closely related to *A. adloffi* species group and to *A. viarius*.

Key-words: Cyprinodontiformes - Rivulidae - *Austrolebias* - Uruguay basin - Los Patos System.

INTRODUCTION

At least seven species of the annual fish genus *Austrolebias* (Rivulidae) are endemic of the Los Patos-Merín system, southern Brazil and eastern Uruguay (Costa & Cheffe, 2001; García *et al.*, 2000). Most of them are distributed in lowlands and wetlands associated to that drainage (Costa & Cheffe, 2001; Azpelicueta & García, 2001), between 0 and 50 meters above sea level. However, at least two species, *A. cyaenus* (Amato, 1987) and *A. vazferreirai* (Berkenkamp *et al.*, 1994), inhabit "highland" localities of that system, between 50 and 100 meters above sea level. Furthermore, they can also be found in temporary ponds of the Río Negro drainage, Uruguay (Berkenkamp *et al.*, 1994; Azpelicueta & García, 2001).

In the present article, we describe a new species of *Austrolebias* with a disrupted distribution that includes "highland" localities from both drainages, Laguna Merín and Río Negro (Río Uruguay basin). We also perform chromosomal studies to contribute with the diagnosis of the populations under study.

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MATERIAL AND METHODS

Measurements were taken with digital calliper following Costa (1988), except peduncle length that was measured from the vertical through last anal fin ray to posterior margin of hypural plate and snout length which was measured from anterior tip of upper lip to a vertical through anterior eye margin.

Measurements were expressed as percentages of SL, or other lengths as indicated. Vertebral counts included the composed caudal centrum as one element. Cephalic neuromasts nomenclature followed Costa (2001). Osteological preparations were made following Dingerkus & Uhler (1977).

In the cytogenetics analysis, metaphases were studied following the protocols of Kligerman & Bloom (1977), with modifications by Bertollo *et al.* (1978). Better metaphase preparations of spleen, kidney, and gill epithelium were selected under a microscope Nikon Microphot-Fx with inmersion objective. Photographs were taken with Kodalith (25 asas). Chromosome nomenclature followed Levan *et al.* (1964) and Denton (1973) for fishes. Based on arm relation (AR), chromosomes were classified as metacentric (M), submetacentric (S), subtelocentric (ST) and acrocentric (A). The diploid number (2n) and the number of chromosome arms (FN) were also established. Nucleolar Organizer Regions (NORs) were identified with silver-staining method by Howell & Black (1980) and constitutive heterochromatic C-band regions were detected according to Sumner (1972). Meiotic studies were performed in male individuals to corroborate haploid number (N) following standard protocols (Kligerman & Bloom, 1977).

The examined specimens were deposited in Muséum d'histoire naturelle de Genève (MHNG) and Vertebrate Collection of Facultad de Ciencias, Montevideo, Uruguay (ZVC-P). Tissues and voucher of *A. arachan* used for chromosomic analysis are deposited in the Sección Genética Evolutiva Facultad de Ciencias, Montevideo, Uruguay (FCMVD-GP) with the numbers 657-661, 775-781, and 1130-1132 (all from Departamento de Cerro Largo, Parque Municipal, Melo city).

Comparative material (SL in mm). *Austrolebias adloffi* Ahl: ZVC-P 724, 25 ex., (8 C&S), 30.75-38.26 mm, Brazil, Rio Grande do Sul, Niteroi, pond into the town; ZVC-P 747, 25 ex., 26.46-29.55 mm, Brazil, Rio Grande do Sul, pond close to Rio Gravataí; ZVC-P 854, 16 ex., 26.00-35.10 mm, Brazil, Rio Grande do Sul, Niteroi, pond close to Rio Gravataí; ZVC-P 855, 13 ex., 27.85-33.70 mm, Brazil, Rio Grande do Sul, Niteroi, Villa Rio Branco; MCP 10288, 12 ex., 38.95-54.29 mm, Brazil, Rio Grande do Sul, Canoas, Rio dos Sinos; MCP 15040, 2 ex., 32.00-39.00 mm, Brazil, Rio Grande do Sul, Rio Gravataí; MCP 10933, 20 ex., 29.37-36.92 mm, Brazil, Rio Grande do Sul, Alvorada, rio, Rio Gravataí; UMMZ 168844, 4 ex., 37.25-50.75 mm, Brazil, Canoas; UMMZ 181681, 13 ex., 37.50-44.55 mm, Brazil, vicinity of Porto Alegre; UMMZ 181682, 11 ex., 32.60-40.70 mm, Brazil, vicinity of Porto Alegre. *Austrolebias bellottii* Steindachner: ZVC-P 876, 61 ex. (19 C&S), 25.20- 46.93 mm, Uruguay, Colonia, Carmelo, pond close to Río Uruguay. *Austrolebias melanoorus* Amato: ZVC-P 4322, 10 ex. (2 C&S), 29.82-38.00 mm, ZVC-P 4323, 10 ex., 26.74-44.64 mm, Uruguay, Tacuarembó, Pueblo Ansina, pond close to Río Tacuarembó 10 ex. (2 C&S). *Austrolebias vandenbergi* Huber: ANSP 175282, 16 ex., 28.73-47.71 mm, Paraguay, Boquerón, pond along road Filadelfia-Teniente Montaña; ANSP 175289, 20 ex., 37.30-55.10 mm, Paraguay, Boquerón, pond along road Filadelfia-Teniente Montaña; ANSP 175290, 25 ex., 24.88-47.44 mm, Paraguay, Boquerón, pond along road Filadelfia-Teniente Montaña. *Austrolebias viarius* Vaz-Ferreira, Sierra & Paulette: ZVC-P 525. 50 ex. (8 C&S), 28.63-49.63 mm, Uruguay, Rocha, pond in the vicinity of A° Valizas; ZVC-P 596, 14 ex. (2 C&S), 27.57-41.67 mm. Uruguay, Rocha, pond next to A° Valizas.

RESULTS

Austrolebias arachan sp. n. Figs 1, 2, Tables I, II

Cynolebias uruguayensis Nion *et al.*, 1994, *nomen nudum*.

Holotype. ZVC-P 4336, male, 38.6 mm SL, Departamento de Cerro Largo, pond close to Route 7 and Arroyo Chuy, Laguna Merín basin, Uruguay, col. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000.

Paratypes. ZVC-P 4574, 6 males (1 C&S), 31.9-43.7 mm SL, 8 females (1 C&S), 24.8-31.3 mm SL, collected with the holotype. ZVC-P 4314, 2 males, 22.9-24.9 mm SL, 1 female 21.6 mm SL, Departamento de Cerro Largo, pond close to Melo city, road to the airport, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000. ZVC-P 4317, 4 males (2 C&S), 26.0-29.5 mm SL, 4 females (2 C&S), 23.2-28.3 mm SL, Departamento Cerro Largo, pond at Parque Municipal, Melo city, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000. ZVC-P 4329, 4 males (all C&S), Departamento de Cerro Largo, pond close to Route 26 and Negro River, Uruguay, coll. M. Loureiro, G. Yemini, C. Hernández, October 1999. ZVC-P 4331, 14 males (3 C&S), 26.9-33.8 mm SL, 7 females, 24.7-33.0 mm SL, Departamento de Cerro Largo, pond close to Route 26 and Río Negro, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000. ZVC-P 4332, 10 males, 23.6-28.2 mm SL, 5 females, 16.4-22.7 mm SL, Departamento de Cerro Largo pond close to Melo city, road to the airport, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000. ZVC-P 4333, 5 males (3 C&S) 28.5-35.6 mm SL, 14 females (5 C&S), 24.5-35.6 mm SL, Departamento de Cerro Largo pond close to Melo city, road to the airport, Uruguay, coll. M. Loureiro, G. Yemini, C. Hernández, October 1999. ZVC-P 4335, 2 males, 30.95-32.45 mm SL, 4 females, 26.3-31.9 mm SL, Departamento de Tacuarembó, Route 26, km 331 pond next to the road, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000. ZVC-P 4528, 3 males (1 C&S), 25.0-29.4 mm SL, 3 females (1 C&S), 23.6-25.9 mm SL, Departamento de Cerro Largo, a pond at Parque Municipal, Melo city, Uruguay, coll. G. García, July 2000. MHNG 2641.88, 3 males, 30.58-33.89 mm SL, 3 females, 24.53-29.38 mm SL, Departamento de Cerro Largo, pond close to Route 26 and Río Negro, Uruguay, coll. M. Loureiro, F. Teixeira, A. D'Anatro, L. Bocardi, September 2000.

Diagnosis. *Austrolebias arachan* is distinguished by the following combination of characters: medium size body (no more than 45.8 mm SL); dorsal-fin insertion anterior to anal-fin origin; long dorsal- and anal-fin bases; male body dark brown to black with light yellow narrow vertical bands, dorsal fin dark brown with vertically elongated light yellow dots on basal half, yellowish brown without dots on distal half; proximal half of anal fin dark brown with vertically elongated light blue dots, distal half light blue without dots; caudal fin light blue with light yellow dots proximally, hyaline on distal fourth; pectoral fin light grey with the ventral margin black. Males with contact organs on scales of flanks, specially distributed over anal fin and around pectoral-, pelvic-, and caudal-fin bases; contact organs on distal third of second to fifth anal fin rays and on three or four uppermost pectoral fin rays distally. Anal fin of females with anterior rays elongated, forming a triangular-shaped fin. Pelvic fins joined to each other at their bases or by an epithelial membrane covering completely or partially (one third) of inner edge in both fins. Two to four parietal neuromasts, sometimes continuous with supra-orbital series. Three pectoral radials in 92 % of individuals examined, four radials in the remaining 8 %.

Description. Meristic and morphometric values are presented in tables I and II respectively. Dorsal profile of head straight or slightly concave; dorsal profile strongly convex from rear of head to dorsal-fin origin, less convex in females; straight below dorsal fin, and straight or scarcely concave on caudal peduncle. Ventral profile arched

from tip of lower jaw to end of anal-fin. Maximum body depth at pelvic-fin origin (Fig. 1); females with lower body.

Dorsal fin with 21-26 rays in males; in females, 16-20. Dorsal-fin tip of males rounded, acute in females. Second proximal pterygophore of dorsal fin through a vertical between vertebrae 7 and 8 in males; through vertebrae 9 and 11 in females. Anal fin with 22-27 rays in males, 17-22 in females. Anal-fin tip rounded in males; anterior rays elongated, forming a triangular fin in females, ratio between longest anal-fin ray/anal-fin base 0.76-1.21 (mean 0.94). Anal-fin origin through a vertical between 2nd to 5th dorsal-fin rays in males and females. Caudal fin with distal margin slightly rounded in males, with 20-29 rays; caudal fin rounded with 20-26 rays in females. Caudal-fin skeleton supported by last three or four vertebrae. Pectoral fin with 11-13 rays in males, 10-14 in females; pectoral-fin margin rounded, with middle rays longer; tip of pectoral fin reaching bases of 2nd to 6th anal-fin rays in males, and half or two thirds of pelvic-fin length of females. Pelvic fin with 4-7 rays, covering urogenital papilla and surpassing 3 to 5 anal-fin ray bases in males, scarcely surpassing anal-fin origin in females; distal fin margin rounded. Pelvic fins joined to each other at their bases or joined by an epithelial membrane covering completely or partially (one third) of inner edge in both fins.

Total number of vertebrae 27-30, number of precaudal vertebrae 11-13; second vertebrae pre-zygapophysis poorly developed; post-temporal ventral process variably developed, process reduced or absent in 47 % of the individuals, in rest process moderate or well developed.

Scales cycloid, 27-34 on lateral series; transverse scales 11-18; scales around caudal peduncle 14-20; 1-3 series over caudal fin. Suborbital and postorbital regions scaled.

Contact organs on scales placed over anal-fin base, many contact organs distributed around pectoral and pelvic fin bases, peduncular area completely covered by contact organs. Contact organs on distal half of uppermost pectoral fin rays 1-3 and on distal third of anal fin rays 2-6.

Supraorbital neuromasts 13-22, parietal neuromasts 2-4. Ventral gill rakers 7-11, dorsal gill rakers 2-4. Basihyal slightly widened, basihyal cartilage 50-60 % of total basihyal length. Two to 4 teeth in second pharyngobranchial. Fourth ceratobranchial with 1 or 2 series of teeth mono-, bi- or multicuspidate. Dermosphenotic absent.

Coloration in life: Male*:* Body dark brown with transverse well defined narrow light yellow bands, between those bands may appear some poorly defined bands with the same coloration. Opercular region bluish. Suborbital and supraorbital bands black. Dorsal fin dark brown with vertically elongated light yellow dots in the proximal half, yellowish brown without dots in the distal half; proximal half of anal fin dark brown with vertically elongated light blue dots, distal half light blue without dots; caudal fin light blue with light yellow dots proximally, hyaline in distal fourth; pectoral fin grey with lower margin black; pelvic fin bluish.

Female: Pale brown body, with darker brown irregular dots or stripes on flanks. Dorsum darker than rest of body. Opercular region yellowish green iridescent. Suborbital band faint, supraorbital absent. Dorsal and anal fins proximally covered by

FIG. 1

Austrolebias arachan, female and male, non-preserved specimens collected in a pond at Parque Municipal, Melo city, Uruguay (one of the paratypes localities).

FIG. 2

Karyogram of *Austrolebias arachan*, somatic cells stained with Giemsa. Diploid number 2n= 48, NF= 60. Scale= 10 mm.

irregular black to brown dots or diffuse vertical bands or completely hyaline; most specimens with dots on basal one third of caudal fin; a few specimens, more or less one third of them, without dots on caudal fin. Pectoral fin hyaline; pelvic fin light grey.

Cytogenetic analysis. The mitotic metaphase studies from 15 individuals (ten males and five females) showed 2n=48, NF=60 and gonial meiotic cells from testis N=24. All individuals with following chromosome formula: three pairs of biarmed chromosomes of submetacentric (SM) type, and 23 pairs of ST-A chromosomes (Fig. 2); three pairs of large acrocentric chromosomes; two Ag-NORs placed at terminal position in small arms of first SM chromosome pair (see arrows, Fig. 2). C-bands lo-

TABLE I. Meristic data of *Austrolebias arachan* from Laguna Merín and Río Negro basins. Holotype and minimum, maximum, and mean of 90 paratypes.

		Males (n= 45)		Females (n= 45)	
	Holotype	min-max	mean	min-max	mean
Dorsal fin rays	22	21-26	23.6	16-20	18.0
Anal fin rays	23	22-27	24.1	17-22	19.8
Caudal fin rays	23	20-29	23.4	20-26	23.3
Pectoral fin rays	13	11-13	12.1	10-14	12.1
Pelvic fin rays	6	4-6	5.4	5-7	5.6
Lateral scales	31	27-34	30.4	28-34	30.5
Transverse scales	14	11-18	14.5	12-16	13.3
Peduncle scales	18	14-20	16.8	14-18	16.2
Predorsal scales	19	14-24	19.9	19-30	22.0
Supraorbital scales	4	0-4	1.8	0-4	20.7
Supraorbital neuromast series	21	13-24	20.7	15-27	2.0

TABLE II. Morphometrics of *Austrolebias arachan* from Laguna Merín and Río Negro basins. Holotype and maximum, minimum, and mean of 86 paratypes in percentage of SL.

		Males (n= 39)		Females (n= 47)	
	Holotype	min-max	mean	min-max	mean
Standard length	38.6	22.9-45.8	30.2	16.4-42.4	26.7
Predorsal length	49.9	47.5-54.1	50.3	51.8-62.0	58.4
Basidorsal length	43.3	38.3-48.1	43.2	25.9-35.7	29.4
Preanal length	51.2	49.3-59.0	53.2	55.2-65.9	60.2
Basianal length	38.2	33.8-48.0	41.4	21.5-31.0	24.9
Prepelvic length	49.6	43.5-49.6	46.9	46.9-57.8	51.8
Body depth	39.0	30.6-42.1	35.2	25.6-37.1	31.0
Peduncle depth	13.6	11.2-15.9	13.5	9.4-14.2	12.3
Peduncle length	12.7	7.7-15.3	10.4	14.9-22.6	18.6
Pectoral fin length	23.9	10.2-27.9	23.6	20.9-30.1	25.4
Pelvic fin length	9.1	7.9-13.3	9.8	9.4-14.0	11.7
Head length	34.2	28.9-34.5	32.2	28.4-36.9	32.4
Head width	59.4	49.7-65.7	57.3	50.2-70.5	59.0
Head depth	93.9	79.5-106.1	88.7	71.9-98.7	81.5
Eye diameter	24.1	23.8-32.9	27.8	23.9-37.9	28.8
Interorbital length	41.7	37.7-50.4	44.3	35.0-48.7	41.9
Snout length	19.7	15.9-24.3	19.9	11.6-23.3	19.1

cated at centromeric and telomeric positions in some chromosome pairs. Large heterochromatic blocks of C-bands occuring at interstitial regions of extra large ST-A type.

Etymology. The name *arachan*, originally refers to native inhabitants from the vicinity of Porto Alegre city and Laguna de los Patos system (Brazil). At present, it refers to people from Departamento de Cerro Largo (Uruguay), where several populations of the new species of *Austrolebias* occur.

Distribution. The new species is known from localities of the upper Río Tacuarí drainage (Laguna Merín basin), and upper Río Negro drainage (Río Uruguay basin); all localities are between 50 and 100 meters above sea level (Fig. 3).

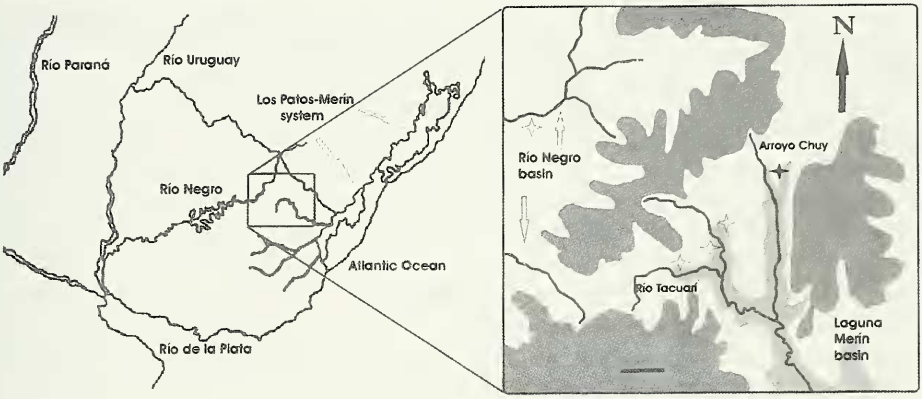

FIG. 3

Geographical distribution of *Austrolebias arachan*. Black star: type locality; white stars: paratypes localities. Dark grey areas represent Cuchilla Grande (more than 150 m above sea level), white areas represent land between 150 and 100 m above sea level, ligth grey areas represent land between 100 and 50 m above sea level. Scale bar= 10 km.

DISCUSSION

Nion *et al.* (1994), in a meeting occurred in Montevideo (Uruguay), presented an abstract entitled "Dos nuevas especies del género *Cynolebias* Steindachner, 1876 (Actinopterygii, Aplocheilidae) del Uruguay". Those authors recognized two new species but they never described them, neither in the abstract nor in other subsequent paper. Therefore, the name *Cynolebias uruguayensis*, used in that abstract for a taxon with similar distribution of the new species here described, became a *nomen nudum*. Furthermore, the material used for that record is lost and this taxon was never described until the present paper.

Austrolebias arachan is related to a group of medium size species that present the anterior anal-fin rays of females elongated (Costa, 2002), pelvic fins joined to each other in different degrees, and two to four parietal neuromasts. The species included in this group are: *A. bellottii*, *A. melanoorus*, *A. vandeubergi*, *A. viarius*, and the *A. adloffi* species group, in which *A. adloffi*, *A. charrua*, *A. uiniuano*, *A. nigrofasciatus* are included (Costa & Cheffe, 2001; Costa, 2002). *Austrolebias arachan* also shares with all the species mentioned, except *A. viarius*, the presence of pectoral fin with ventral margin black. The pigmentation pattern in the body male of *A. arachan* easily distinguishes it from most of the species aforementioned, except from *A. viarius*. However, *A. viarius* is distinguished from males of *A. arachan* by the darker coloration of body and fins (body dark brown to black vs. brown orange, Vaz Ferreira *et al.*, 1964), a relatively larger dorsal fin base (mean 43.2 vs. 38.8 % of SL), and a relatively narrower head (mean 57.3 vs. 62.2 % of HL); both sexes of *A. arachan* and *A. viarius* are also differentiated by the absence in the former of black dots on the flanks. *Austrolebias arachan* is further distinguished from *A. adloffi* species group by the absence of vertically arranged black dots in the caudal peduncle. Furthermore, the proportion of individuals with reduced postemporal process is higher in *A. arachan* (45 %) than in all species abovementioned (less than 30 %).

Parsimony analysis based on 27 data set of cyt-b sequence including eighteen species of *Austrolebias* and six other Rivulidae genera (García *et al.*, 2002) confirmed that *A. arachan* (*C.* sp GG-661, in that article) is closely related to the *A. adloffi* species group, and to *A. viarius*. Also, cytogenetic data revealed that *A. arachan* presents a generalized karyotype composed by 48 chromosomes of predominantly ST-A chromosome type as was reported for other Cyprinodontiforms groups (Ebeling & Chen, 1970) and other taxa within *Austrolebias* (García *et al.*, 1993, 1995, 2002). However, *A. arachan* has distinctive karyological characters from those of *A. adloffi*, and *A. viarius*. *Austrolebias arachan* has three pairs of SM chromosome type, while *A. adloffi* bears one pair of biarmed chromosomes and *A. viarius* one or two pairs. Furthermore, *A. arachan* presents three extra-large ST-A chromosomes as markers. This type of chromosomes were found in species included in other clades of *Austrolebias* such as *A. alexandri* and *A. gymnoventris* but they were not found in *A. viarius* and *A. adloffi* (García *et al.*, 1995).

Austrolebias arachan shows two NORs at terminal positions of the first biarmed chromosomes. The variability in number and position of NOR regions observed in this taxon is very low in comparison with the high variability detected in previous studies within the genus *Austrolebias* and among sister taxa of the clade (García *et al.*, 1993, 1995, 2001). Peculiar large heterochromatic blocks of C-bands were detected at interstitial regions of the extra large ST-A type; this is an additional different cytogenetic character in *A. arachan*, not found in *A. viarius* and *A. adloffi*.

Austrolebias arachan, together with *A. vazferreirai,* has a unique geographical distribution within the genus, because no other species is found in localities of the two different drainages of the Río Negro and the Laguna Merín. In addition, both drainages belong to different ichthyogeographical regions such as the Río de la Plata basin and the Coastal Atlantic rivers (Ringuelet, 1975). The distribution pattern of the new species could be an indicator of an ancient connection between both systems and regions. The Río Negro basin is separated from the Laguna Merín basin by the Cuchilla Grande (up to 300 m above sea level). However, this hills system is interrupted by an area between 150 and 100 meters above sea level, close to *A. arachan* distribution (Fig. 3). This distribution pattern would suggest a relatively ancient origin of these species and also the possibility of dispersion caused by birds (Santamaría & Klassen, 2002). Annual fish present drought resistant eggs which could rise the probability of successful dispersion by this way. However, this kind of phenomena has not been proved for these fish yet.

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